Giornale Italiano di Endodonzia (2016) **30**, 89—95

Società Italiana di Endodonzia

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ORIGINAL ARTICLE/ARTICOLO ORIGINALE

The influence of antibiotics on the physical properties of endodontic cements

Influenza dell'utilizzo di soluzioni antibiotiche sulle proprietà meccaniche di cementi endodontici

M.A. Saghiri [a,*], A. Asatourian [b], J. Orangi [b], J.W. Soukup [c], J.L. Gutmann [d], F. Garcia-Godoy [e], N. Sheibani [a]

[a] *Departments of Ophthalmology & Visual Sciences and Biomedical Engineering, University of Wisconsin School of Medicine and Public Health, Madison, WI, USA*
[b] *Sector of Angiogenesis and Regenerative Surgery, Dr. Hajar Afsar Lajevardi Research Cluster, Shiraz, Iran*
[c] *Department of Surgical Sciences, School of Veterinary Medicine, University of Wisconsin, Madison, WI, USA*
[d] *Department of Restorative Sciences, Texas A&M University College of Dentistry, Dallas, TX, USA*
[e] *University of Tennessee Health Science Center, Memphis, TN, USA*

Received 17 May 2016; accepted 13 September 2016
Available online 2 November 2016

KEYWORDS
Antibiotic;
Chlorexidine;
Microhardness;
Push-out strength;
MTA.

Abstract
Aim: To evaluate the influence of Metronidazole, Minocycline and Ciprofloxacin as a mixture or individually and of chlorhexidine on the push-out bond strength and surface microhardness of calcium silicate cements of differing particle size.
Methodology: 120 extracted adult human premolars were decoronated and 2 mm dentin slices were prepared. Specimens were divided equally into the following groups: normal saline and CHX, Metronidazole, Minocycline, Ciprofloxacin, and combination of Metronidazole, Minocycline and Ciprofloxacin. The specimens were irrigated with solutions and filled with endodontic cements. In the second part, the endodontic cements were mixed, placed in plastic tubes

* Corresponding author at: Departments of Ophthalmology & Visual Sciences and Biomedical Engineering, University of Wisconsin School of Medicine and Public health, Madison, WI, USA.
E-mail: saghiri@wisc.edu (M.A. Saghiri).
Peer review under responsibility of Società Italiana di Endodonzia.

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http://dx.doi.org/10.1016/j.gien.2016.09.003

and then irrigated for 1 or 5 min. Push-out and surface microhardness values were calculated and data were analyzed with three way ANOVA followed by Tukey's post-hoc test.
Results: The normal saline and ciprofloxacin groups showed significantly higher and lower, respectively, push-out bond strength among the experimental groups ($p < 0.001$ for all groups). Nano type cement showed higher push-out bond strength and microhardness than regular one at both time intervals. The mixture of antibiotics had significant effects on the push out and microhardness of calcium silicate cement.
Conclusions: Nano particle MTA resisted more than the conventional MTA to the effect of the irrigating solution and antibiotics in both hardness and push-out strength. Furthermore, the results of microhardness were consistent with the push-out strength in most cases. The microhardness test may be employed as a complimentary test to evaluate push-out strength of dental cements.

PAROLE CHIAVE
Antibiotici;
Clorexidina;
Microdurezza;
Resistenza alla dislocazione;
MTA.

Riassunto
Obiettivo: valutare l'influenza dell'uso di metronidazolo, minociclina e Ciprofloxacina come miscela di antibiotici o singolarmente e della clorexidina sulla forza di legame e la microdurezza superficiale di cementi di silicato di calcio di diversa granulometria.
Metodologia: 120 premolari umani adulti estratti sono stati decoronati e sono state preparate fette di dentina di 2 mm. I campioni sono stati divisi in parti uguali nei seguenti gruppi a seconda della soluzione irrigante utilizzata: soluzione fisiologica e CHX, metronidazolo, minociclina, ciprofloxacina, e una combinazione di Metronidazolo, minociclina e ciprofloxacina. I campioni sono stati irrigati con una di queste soluzioni e riempiti di cemento endodontico e poi sottoposti a test di push-out. Nella seconda parte, i cementi endodontici sono stati mescolati, messi in provette di plastica e poi posti in contatto per 1 o 5 min con le diverse soluzioni prima di essere testati per microdurezza superficiale. I valori push-out e microdurezza superficiale sono stati calcolati e i dati sono stati analizzati con test ANOVA a tre vie seguito dal test post hoc di Tukey.
Risultati: I gruppi trattati con soluzione saline e con la ciprofloxacina hanno mostrato rispettivamenteuna forza di legame significativamente più alta e più bassa tra i gruppi sperimentali ($p < 0.001$). Il cemento di tip nanao ha mostrato una maggiore forza di legame al test push-out e una maggiore microdurezza superficiale rispetto al cemento regolare nei due intervalli di tempo. La miscela di antibiotici ha avuto effetti significativi sulla resistenza alla dislocazione e sulla microdurezza superficiale dei cemento al silicato di calcio.
Conclusioni: Un MTA a nanoparticelle ha resistito amggiormente all'effetto nesativo delle soluzioi irriganti e antibiotiche rispetto al MTA convenzionale sia per quanto riguarda la durezza superficiale che la resistenza al push-out. Inoltre, i risultati di microdurezza superficiali sono risultati correlati alla forza di legame nella maggior parte dei casi. Il test microdurezza può essere impiegato come test complementare per valutare la forza al push-out dei cementi endodontici.

Introduction

Since the introduction of Mineral Trioxide Aggregate (MTA) to the field of dentistry[1] several variables, such as the powder-to-water ratio,[2] mixing technique,[3] humidity and setting time[4], storage temperature[5], and the pH value of applied area[6–8] have been shown to have an impact on different properties of MTA. Likewise, post-setting factors such as thermal fluctuation after cement application can have significant impact on the structural and physical properties of this material.[9] In addition to environmental variables, there are different materials that are used in root canal procedures which can produce similar alterations[10] and may affect biologically root canal treatment and regeneration.[11,12]

Chlorhexidine (CHX) is a cationic based antiseptic that is active against a wide range of microorganisms including aerobic and anaerobic gram-positive and negative bacteria, viruses, molds and yeasts.[13,14] The mutual effect of CHX and MTA cement has been questioned from antibacterial and cytotoxicity characteristics.[10] When 0.12% CHX was mixed with MTA cement, the antibacterial properties of CHX can be beneficial for MTA; however, MTA can increase the cytotoxicity of CHX as a result.[10]

Antimicrobial agents are frequently employed within root canals in form of liquids, pastes or solids. Investigations have confirmed the use of combination of Metronidazole, Minocycline and Ciprofloxacin for sterilization of root canal systems and are now acceptable in clinical practice.[14–16] An *in vitro* study[15] testing the antibacterial efficacy of these drugs alone and in combination indicated that these drugs individually could not completely eliminate bacterial contamination, however the mixture of these antibiotic was able to consistently sterilize all samples. Published studies have claimed that this mixture is able to eradicate the tested microorganisms in vitro.[14]

Nano WMTA is a nano version of WMTA,[17] which in addition to having the nano particle size powder it has different additives to accelerate and amplify its hydration process.[18,19] Apart from some advantages of Nano WMTA, such as lower setting time and higher compressive and push-out strength in comparison with WMTA,[9,18,19] studies have investigated the changes in the physical properties of this cement in different settings.[3,9] Investigations have shown that similar to WMTA, alterations in setting condition can reduce the physical properties of Nano WMTA. However, Nano WMTA withstands these changes better than WMTA.[3,9]

The present study evaluated the effect of disinfectant and antibiotic materials such as CHX gel, Metronidazole, Minocycline, Ciprofloxacin and their combination on the push-out bond strength and micro-hardness of WMTA and Nano WMTA to the dentinal surface. The hypothesis tested was that the use of these agents as a canal irrigating material or intracanal medication, will affect the push-out bond strength and the surface micro-hardness of the cements, which could be deleterious to the desired sealing ability of the MTA materials.

Materials and methods

Antibiotic mixture preparation

Based on a previous study,[15] Metronidazole, Minocycline hydrochloride and Ciprofloxacin (all antibiotics purchased from Sigma—Aldrich, St. Louis, MO) were mixed under aseptic conditions and UV light using a spatula to prepare a mixture with proportions of 1:5:1, respectively, to yield 8.3 g, which was then mixed with a mortar and pestle to create a powder. The powder was added to 50 mL milli Q water and vortexed for 5 min to make a solution.

Push-out bond strength

This study was divided into two parts, the first part being similar to Saghiri et al.[19] Briefly, 120 extracted single-rooted human teeth were selected and sectioned horizontally using a low-speed Isomet diamond saw (Buehler, Lake Bluff, IL, USA) at the mid-root portion to prepare 2 mm thick dentin slices. The canal spaces were instrumented by using #2 through #5 Gates-Glidden burs (Mani, Tochigi, Japan) to form 1.3 mm diameter standardized spaces. Subsequently, debris and smear layer removal was done by immersion in 17% EDTA, then in 5.25% NaOCl for one minute in each. The specimens were divided into the following groups: Normal Saline, CHX (12 specimens irrigated for 1 min and randomly filled with WMTA or Nano WMTA), Metronidazole, Minocycline, Ciprofloxacin, and the combination of the antibiotics (12 specimens irrigated for 1 min randomly divided into two groups and filled with WMTA or Nano WMTA and 12 specimens irrigated for 5 min and similarly prepared).

Dentin slices were irrigated with distilled water, dried and irrigated with 3 mL of normal saline, CHX, Metronidazole, Minocycline, Ciprofloxacin, and the combination of the antibiotics for 1 or 5 min. After irrigation, the canals were filled with WMTA (Angelus Dental Industry Products, Londrina, Brazil) or Nano WMTA (Kamal Asgar Research Centre). Cements were mixed according to the manufacturers' instruction and transferred into the canal spaces using a manual MTA carrier and packed using a hand compactor. A piece of gauze soaked in synthetic tissue fluid, prepared from 1.7 g of KH_2 PO_4, 11.8 g of Na_2HPO_4, 80.0 g of NaCl, and 2.0 g of KCl in 1 L of H_2O (pH 7.4), was placed on each sample and all dentin slices were incubated at 37 °C with 98% humidity for 3 days. The gauze was changed every 6 h to preserve the stability of the setting condition for the cement.

The push-out bond strength was evaluated using Universal testing machine (Sintech MTS, Eden Prairie, MN, USA). Samples were fixed on a metal slab with a central hole. A stainless steel plunger with 1 mm diameter was used to apply the downward compressive load with crosshead speed of 1 mm/min on the cement materials.

Vickers hardness test

The second part of the study was similar to Saghiri et al.[20] Briefly, 120 plastic tubes were divided into experimental groups same as the first part of the study. Subsequently, WMTA and Nano WMTA cements were mixed and packed by using a hand compactor into the plastic tubes as is shown in Fig. 1. Five minutes after packing the surface of the cements were exposed to one droplet of the following material: normal saline, CHX, Metronidazole, Minocycline, Ciprofloxacin, and the combination of the antibiotics. The droplets were cleaned 1 min or 5 min after exposure and the specimens were incubated for 24 h. The specimens were then ground as reported by Saghiri et al.[20] and prepared for Vickers hardness test.

Type of failure

Specimens used to test the 1 min time interval push-out bond strength were also evaluated to determine the type of bond

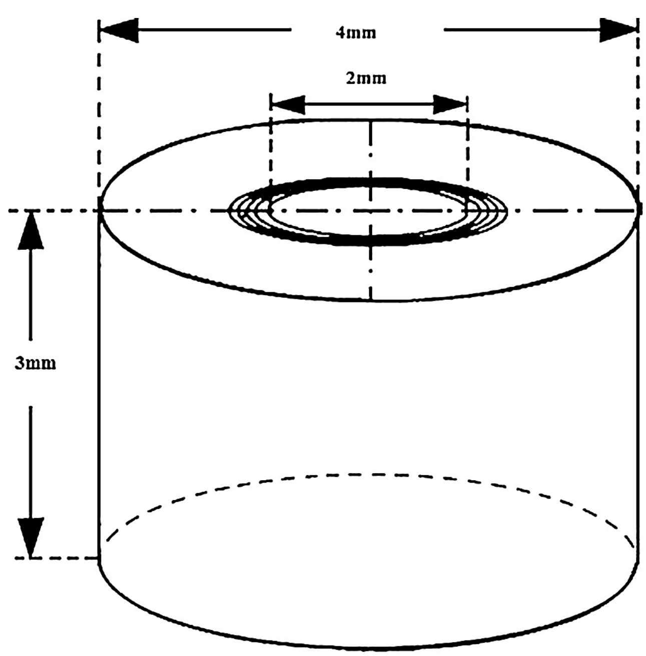

Figure 1 Tube used to determine surface hardness of experimental materials.

failure. Samples were analyzed with a digital camera attached to a stereomicroscope (Olympus, SZM9) at 16× magnification. The type of bond failure was determined by the area of cement remaining on the surface of canal dentin and then categorized as adhesive, mixed or cohesive. Bond failure analysis was performed by a single observer who was blind to the experimental groups.

Statistical analysis

Data showed normal distribution using a Kolmogorov—Smirnov test. To assess the impact of materials, antibiotics and time on the dependent variables push-out strength and hardness and their interactions three-way ANOVA followed by Tukey's post hoc was performed.

Results

Push-out strength

Mean ± SD of the data is presented in Fig. 2A. The Material × Antibiotic × Time three-way interaction was not found to be statistically significant, ($F = 0.70$, $p = 0.554$). Neither the Material × Antibiotic, Material × Time, Antibiotic × Time interactions were found to be statistically significant ([$F = 0.564$, $p = 0.728$], [$F = 0.056$, $p = 0.813$],

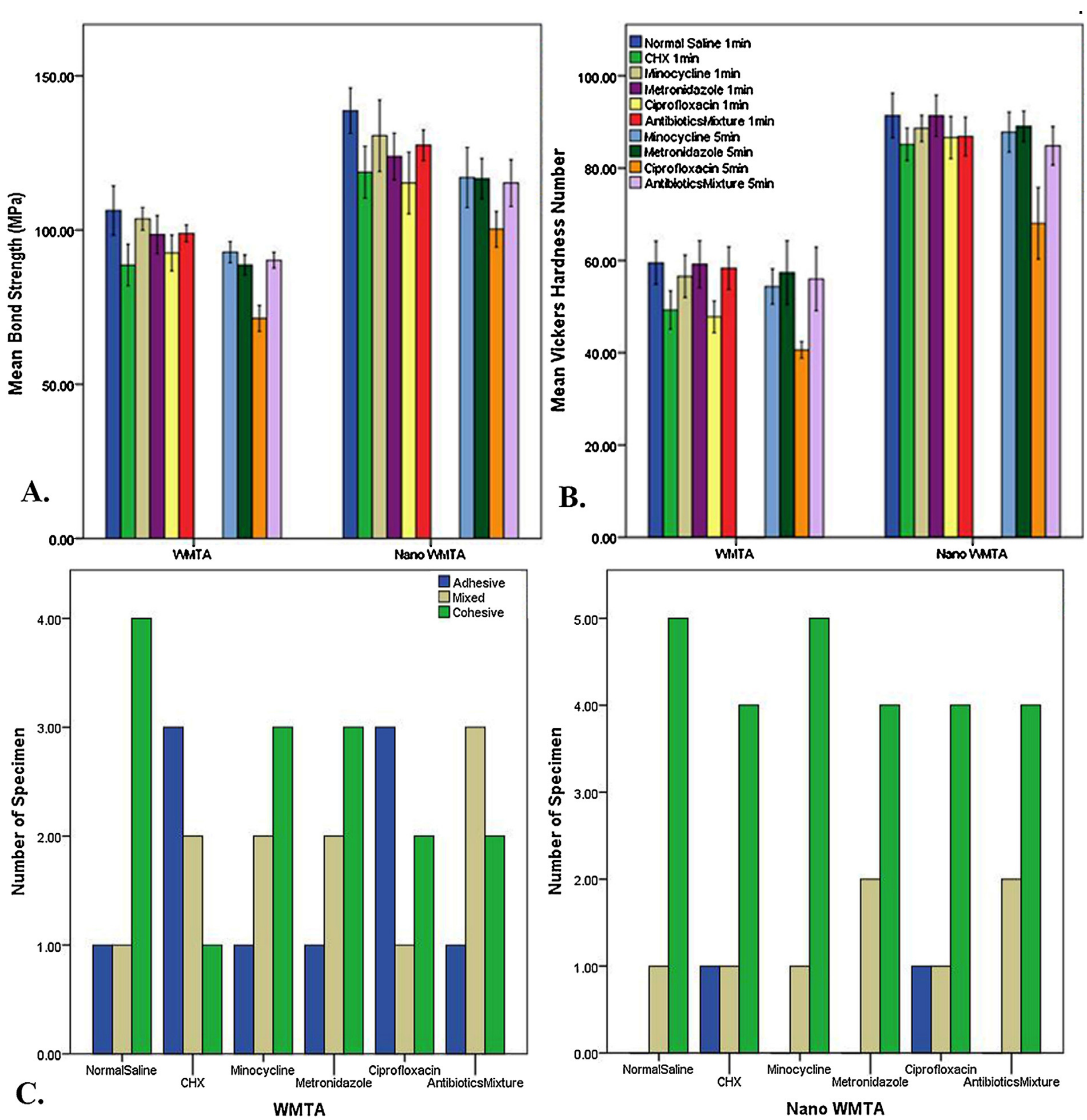

Figure 2 Mean ± SD of (A) Push-out Strength and (B) Vickers Hardness Number. (C) Failure mode in dentin—cement interface. Adhesive failure: less than 25% cement remnant on dentinal surface. Mixed adhesive/cohesive failure: 25—75% cement remnant on dentinal surface cohesive failure: More than 75% cement remnant on dentinal surface. Left. WMTA, Right. Nano WMTA.

[$F = 2.239$, $p = 0.088$] respectively). Statistically significant main effect was found for all factors (Material [$F = 463.533$, $p < 0.001$], Antibiotic [$F = 25.325$, $p < 0.001$], Time [$F = 79.264$, $p < 0.001$]). Simple main effects analyses, followed by post-hocs, were performed on the independent factors. For instance, the normal saline group showed statistically significant higher push-out strength compared to the other experimental groups ($p < 0.001$ for all groups), and ciprofloxacin showed significantly lower push-out strength among other groups ($p < 0.001$ for all groups). Nano WMTA showed significantly higher push-out strength than WMTA.

Vickers hardness test

Mean $\pm$ SD of the data is presented in Fig. 2B. The Material $\times$ Antibiotic $\times$ Time three-way interaction was not found to be statistically significant ($F = 2.424$, $p = 0.070$). Of the two-way interaction terms tested, the Antibiotic $\times$ Time was found to be statistically significant ($F = 8.184$, $p < 0.001$). In 1-min interval, the CHX and Ciprofloxacin groups showed significantly lower hardness compared to the other groups ($p < 0.001$, $p = 0.006$, $p < 0.001$ and $p = 0.006$ for normal saline, Minocycline, Metronidazole and antibiotic mixture respectively). Furthermore, in the 5-min time interval, ciprofloxacin also showed significantly lower hardness ($p < 0.001$). Statistically significant main effect was found for all factors (Material [$F = 1296.127$, $p < 0.001$], Antibiotic [$F = 27.212$, $p < 0.001$], Time [$F = 23.457$, $p < 0.001$]). Nano WMTA showed significantly higher hardness than WMTA in both time intervals.

Type of failure

The stereo microscope observations characterized the modes of bond failure of the experimental groups. The results are presented in Fig. 2C and D.

Discussion

The use of antibiotics has been widely accepted in endodontics. Despite some advance in this area, the effect of antibiotics on the physical properties of root end filling materials need future evaluation. The present study is an attempt to evaluate the influence of different antibiotics, including Ciprofloxacin, Metronidazole and Minocycline, on the physical properties of a well-known root end filling material WMTA.

Several studies have evaluated the physicochemical changes of these cements in different situations or against environmental variables.[3–9,21,22] The push-out test is closely related to the sealing characteristics of calcium silicate based cements.[21] Parameters such as pin diameter, specimen thickness, and the elastic modulus of tested material may affect the bond strength values.[23] Therefore, the dentin slices were 2 mm in thickness,[23] and the plunger used for applying the forces in push-out test was 1 mm in diameter that was also chosen to be 0.85 times smaller than the tested materials.[23] The push-out test methodology, samples preparation, and the concentrations of antibiotic mixture were selected as previously reported.[4,7,19,24] Moreover, because 2% CHX is an effective solution for disinfecting the root canal system,[25–27] this irrigating solution was also used here.

Our results indicated that the samples which were exposed to CHX showed significantly lower push-out strength values in 1 min time interval compared with other experimental groups except Ciprofloxacin. In addition, Ciprofloxacin showed significantly lower push-out bond strength values in both time intervals. These results were not consistent with previous studies, that indicated 2% CHX did not impact the push-out bond strength of MTA-dentin significantly.[27,28] This difference might be explained by the methodology used by these authors, as they noted a decrease in bond strength values of MTA-dentin in samples exposed to 2% CHX and 5.25% NaOCl. Furthermore, in other previous studies the effect of 0.12% CHX was tested on the antimicrobial activity of MTA cement.[10,29] Although 0.12% CHX can enhance the antimicrobial activity of MTA cement, the cytotoxicity of CHX was increased[10] and CHX produced some alterations on the dentinal surface.[29] These structural changes produced by CHX seem to be an important factor in decreasing the push-out bond strength value of tested calcium silicate-based cements.

The comparison between WMTA and Nano WTMA experimental groups showed that the push-out values in Nano WMTA subgroups were remarkably higher than WMTA samples. These outcomes were consistent with previous studies' results[9,19] where Nano WMTA showed higher dislodgement force in different tested environments than WMTA. This can be explained by the nano structure of this cement which provides more surface area for reaction between the powder particles and the liquid.[9,19] In addition, the additives of Nano WMTA such as zeolite, tricalcium aluminate, strontium carbonate and calcium sulfate can provide better distribution for powder particles and act as stabilizing agents in different situations.[9,19]

The results of the current study indicated that Nano WMTA has higher hardness than WMTA which was consistent with previous studies.[17,18] Statistical analysis of data revealed that CHX and Ciprofloxacin showed significantly lower hardness compared with other groups. As other authors pointed out, this may contribute to significant interaction between the cements and the environments where the cements hardened.[20,30] According to the present results, the Ciprofloxacin group showed the lowest pH value (pH 3.4) compared with Normal Saline, CHX, Minocycline, Metronidazole and the antibiotic mixture, which results in lower hardness (pH 6.5, 7.5, 4.5, 6.5 and 5.8 respectively). Therefore, the results of the current study illustrated that pH influenced hardness of calcium silicate based cements as other researchers have stated.[31]

It could be inferred from the results, that Nano WMTA with antibiotics mixture showed higher push-out bond strength than WMTA with Normal Saline. Indeed, if using CHX, antibiotics or their mixture is recommended clinically, Nano WMTA with antibiotic mixture could be used instead of the commercial WMTA while having higher push-out bond strength and hardness. This could be due to the nano sizing effect and increasing the surface area of the nano powder. The evaluation of types of bond failure was consistent with the push-out strength values of WMTA and Nano WMTA groups. In Nano WMTA samples the cohesive type of failure was more than WMTA specimen. This difference can be explained by the higher dislodgement force of Nano WMTA samples that showed more resistance against the forces during push-out test. This finding is attributed to the better interlocking of Nano WMTA cement inside the dentinal structure[3,19] and can lead to cohesive type of failure, which is obviously seen through the Nano WMTA cement remnant on the surface of root canal dentin.

Conclusions

- Antibiotic solutions had significant influence on push-out bond strength and surface microhardness of calcium silicate cements.
- Nano particles resisted more than regular cement to irrigating solution and antibiotics in both terms of surface hardness and push-out bond strength.
- In most groups, surface microhardness was consistent with push-out results. Consequently, the microhardness test could be a complimentary test for the evaluation of push-out bond strength of dental cement.

Conflict of interest

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors. M Ali Saghiri holds a US patent for Nano Cement.

Acknowledgments

This publication is dedicated to the memory of Dr. Hajar Afsar Lajevardi, a legendry Iranian Pediatrician (1955—2015) who passed away during the writing of this article. We will never forget Dr. H Afsar Lajevardi's kindness and support. She was the prominent clinician-scientist with a particular focus on infectious diseases of children in Iran. The authors also acknowledge the unrestricted award from Research to Prevent Blindness to the Department of Ophthalmology and Visual Sciences, Retina Research Foundation, P30 EY016665, P30 CA014520, EPA 83573701, and EY022883. In addition, we thank Drs. Ali Mohammad Saghiri and Mona Momeni Moghadam for their assistance with the manuscript. Also, none of the supporters have had any role in direction of this project.

References

1. Torabinejad M, White DJ. Tooth filling material and method of use: Google Patents; 1995.
2. Saghiri MA, Asgar K, Lotfi M, Karamifar K, Neelakantan P, Ricci JL. Application of mercury intrusion porosimetry for studying the porosity of mineral trioxide aggregate at two different pH. *Acta Odontol Scand* 2012;**70**:78—82.
3. Saghiri MA, Garcia-Godoy F, Gutmann JL, Lotfi M, Asatourian A. Effects of various mixing techniques on physical properties of white mineral trioxide aggregate. *Dent Traumatol* 2014;**30**:240—5.
4. Gancedo-Caravia L, Garcia-Barbero E. Influence of humidity and setting time on the push-out strength of mineral trioxide aggregate obturations. *J Endod* 2006;**32**:894—6.
5. Saghiri MA, Lotfi M, Joupari MD, Aeinehchi M, Saghiri AM. Effects of storage temperature on surface hardness, microstructure, and phase formation of white mineral trioxide aggregate. *J Endod* 2010;**36**:1414—8.
6. Hashem AAR, Amin SAW. The effect of acidity on dislodgment resistance of mineral trioxide aggregate and bioaggregate in furcation perforations: an in vitro comparative study. *J Endod* 2012;**38**:245—9.
7. Shokouhinejad N, Nekoofar MH, Iravani A, Kharrazifard MJ, Dummer PM. Effect of acidic environment on the push-out bond strength of mineral trioxide aggregate. *J Endod* 2010;**36**:871—4.
8. Saghiri MA, Lotfi M, Saghiri AM, Vosoughhosseini S, Fatemi A, Shiezadeh V, et al. Effect of pH on sealing ability of white mineral trioxide aggregate as a root-end filling material. *J Endod* 2008;**34**:1226—9.
9. Saghiri MA, Asatourian A, Garcia-Godoy F, Gutmann JL, Sheibani N. The impact of thermocycling process on the dislodgement force of different endodontic cements. *Biomed Res Int* 2013;2013.
10. Hernandez E, Botero T, Mantellini M, McDonald N, Nör J. Effect of ProRoot® MTA mixed with chlorhexidine on apoptosis and cell cycle of fibroblasts and macrophages in vitro. *Int Endod J* 2005;**38**:137—43.
11. Saghiri MA, Asatourian A, Sheibani N. Angiogenesis in regenerative dentistry. *Oral Surg Oral Med Oral Pathol Oral Radiol* 2015;**119**:122.
12. Saghiri MA, Asatourian A, Sorenson CM, Sheibani N. Role of angiogenesis in endodontics: contributions of stem cells and proangiogenic and antiangiogenic factors to dental pulp regeneration. *J Endod* 2015;**41**:797—803.
13. Hauman C, Love R. Biocompatibility of dental materials used in contemporary endodontic therapy: a review. Part 1. Intracanal drugs and substances. *Int Endod J* 2003;**36**:75—85.
14. Mehrvarzfar P, Saghiri MA, Asatourian A, Fekrazad R, Karamifar K, Eslami G, et al. Additive effect of a diode laser on the antibacterial activity of 2.5% NaOCl, 2% CHX and MTAD against *Enterococcus faecalis* contaminating root canals: an in vitro study. *J Oral Sci* 2011;**53**:355—60.
15. Hoshino E, Kurihara-Ando N, Sato I, Uematsu H, Sato M, Kota K, et al. In-vitro antibacterial susceptibility of bacteria taken from infected root dentine to a mixture of ciprofloxacin, metronidazole and minocycline. *Int Endod J* 1996;**29**:125—30.
16. Sato T, Hoshino E, Uematsu H, Kota K, Iwaku M, Noda T. Bactericidal efficacy of a mixture of ciprofloxacin, metronidazole, minocycline and rifampicin against bacteria of carious and endodontic lesions of human deciduous teeth in vitro. *Microb Ecol Health Dis* 1992;**5**:171—7.
17. Saghiri MA, Lotfi M, Aghili H. Dental cement composition: US Patent 8,668,770, 2014.
18. Saghiri M, Asgar K, Lotfi M, Garcia-Godoy F. Nanomodification of mineral trioxide aggregate for enhanced physiochemical properties. *Int Endod J* 2012;**45**:979—88.
19. Saghiri MA, Garcia-Godoy F, Gutmann JL, Lotfi M, Asatourian A, Ahmadi H. Push-out bond strength of a nano-modified mineral trioxide aggregate. *Dent Traumatol* 2013;**29**:323—7.
20. Saghiri MA, Lotfi M, Saghiri AM, Vosoughhosseini S, Aeinehchi M, Ranjkesh B. Scanning electron micrograph and surface hardness of mineral trioxide aggregate in the presence of alkaline pH. *J Endod* 2009;**35**:706—10.
21. Parirokh M, Torabinejad M. Mineral trioxide aggregate: a comprehensive literature review—part I: chemical, physical, and antibacterial properties. *J Endod* 2010;**36**:16—27.
22. VanderWeele RA, Schwartz SA, Beeson TJ. Effect of blood contamination on retention characteristics of MTA when mixed with different liquids. *J Endod* 2006;**32**:421—4.
23. Chen W-P, Chen Y-Y, Huang S-H, Lin C-P. Limitations of push-out test in bond strength measurement. *J Endod* 2013;**39**:283—7.
24. Sato I, Ando-Kurihara N, Kota K, Iwaku M, Hoshino E. Sterilization of infected root-canal dentine by topical application of a mixture of ciprofloxacin, metronidazole and minocycline in situ. *Int Endod J* 1996;**29**:118—24.

25. Leonardo M, Tanomaru Filho M, Silva L, Nelson Filho P, Bonifacio K, Ito I. In vivo antimicrobial activity of 2% chlorhexidine used as a root canal irrigating solution. *J Endod* 1999;**25**:167—71.
26. Wang CS, Arnold RR, Trope M, Teixeira FB. Clinical efficiency of 2% chlorhexidine gel in reducing intracanal bacteria. *J Endod* 2007;**33**:1283—9.
27. Yan P, Peng B, Fan B, Fan M, Bian Z. The effects of sodium hypochlorite (5.25%), Chlorhexidine (2%), and Glyde File Prep on the bond strength of MTA-dentin. *J Endod* 2006;**32**:58—60.
28. Guneser MB, Akbulut MB, Eldeniz AU. Effect of various endodontic irrigants on the push-out bond strength of biodentine and conventional root perforation repair materials. *J Endod* 2013;**39**:380—4.
29. Stowe TJ, Sedgley CM, Stowe B, Fenno JC. The effects of chlorhexidine gluconate (0.12%) on the antimicrobial properties of tooth-colored ProRoot mineral trioxide aggregate. *J Endod* 2004;**30**:429—31.
30. Giuliani V, Nieri M, Pace R, Pagavino G. Effects of pH on surface hardness and microstructure of mineral trioxide aggregate and Aureoseal: an in vitro study. *J Endod* 2010;**36**:1883—6.
31. Namazikhah MS, Nekoofar MH, Sheykhrezae MS, Salariyeh S, Hayes SJ, Bryant S, et al. The effect of pH on surface hardness and microstructure of mineral trioxide aggregate. *Int Endod J* 2008;**41**:108—16.